A Green Approach to Encapsulate Proteins and Enzymes Within MOFs: Focusing on proteins location and conformation.

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Laccases (LCs, benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multi-copper oxidases able to oxidize various phenolic compounds. Due to their low substrate specificity, LCs are of potential technological interest in the fields of green chemistry such as bioethanol production.[1] However, the use of free LC is often hampered by several limitations such as: high costs, low operational stability (extreme conditions of T and pH) and difficult recovery and reuse. Most of these drawbacks can be overcome through LC immobilization on solid supports. Recently, a new class of solid material known as Metal-organic frameworks (MOFs), due to their proprieties, such as high porosity and surface area, MOFs have been explored for several applications, like removal of water pollutants,[2] gas adsorption and catalysis,[3]. Recently, MOFs have shown great potential as enzyme immobilization supports [4]. Here, the LC from *Aspergillus sp.* was immobilized within two Lanthanide-based Tb and Gd MOFs (TbBTC and GdBTC) through a one-pot synthesis carried out under mild reaction conditions, e.g. aqueous solution, almost neutral pH, and at room temperature. (Figure 1) The free LC, LC@Tb-BTC, LC@Gd-BTC were also characterized in terms of specific activity, kinetic parameters (K_M and V_{max}) and storage stability in water and acetate buffer.

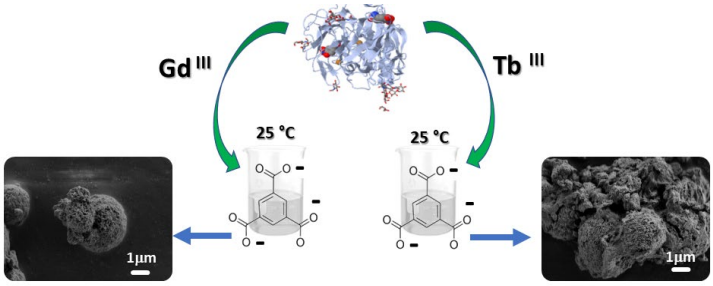

Figure 1 –Schematic preparation of the LC@TbBTC and LC@GdBTC biocatalysts.

Although MOFs synthesized under mild conditions have shown great potential for in situ enzymatic immobilization, the enzyme location and its change in conformation after immobilization within MOFs are still poorly investigated [5]. Due to their low grade of purity and their still unknown structure, the use of commercial enzymes could make the investigation of their location and conformation tricky. Therefore, Bovine serum albumin (BSA), with high purity (> 98%) with a known secondary and tertiary structure, was used as model protein. BSA was immobilized within TbBTC and GdBTC and two different zeolitic imidazolate frameworks (ZIF-zni and ZIF-8). (Figure 2) Pristine materials, and the BSA@MOFs samples were characterized by small/wide angle X-ray scattering analysis, scanning electron microscopy, confocal laser scanning microscopy, thermogravimetric analysis, and micro-FTIR confocal Raman spectroscopy to evaluate the protein location in the materials. Moreover, the secondary structure and conformation changes of BSA due to its immobilization on both ZIF-zni and ZIF-8 were investigated by deconvolution of FTIR spectra. Data showed that BSA is evenly distributed both around and within ZIFs. The crystalline content of BSA increases significantly when the protein is immobilized on both ZIFs.

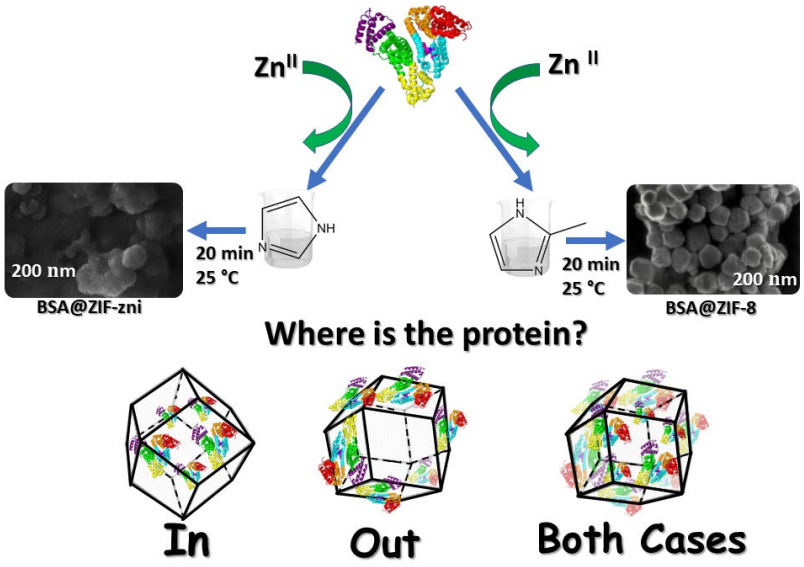

Figure 2 –Schematic preparation of the BSA@ZIF-zni and BSA@ZIF-8 biocatalysts.

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